



- 1. A protein derived from an enterically transmitted non-A/non-B viral hepatitis agent whose genome contains a region which is homologous to a coding region of the 1.33 kb DNA EcoRI insert present in plasmid pTZKF1(ET1.1) carried in E. coli strain BB4 and having ATCC deposit no. 67717.
- 10 2. The protein of claim 1, which is encoded by a complete coding region within said 1.33 kb EcoRI insert.
- 3. A recombinant protein derived from an enterically transmitted nonA/nonB viral hepatitis agent whose genome contains a region which is homologous to a coding region of a DNA molecule having a first sequence (SEQ ID NO.1):

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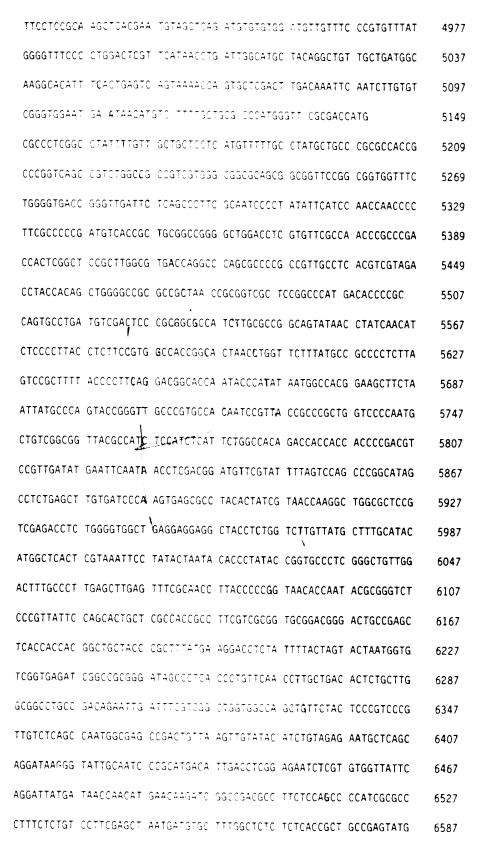


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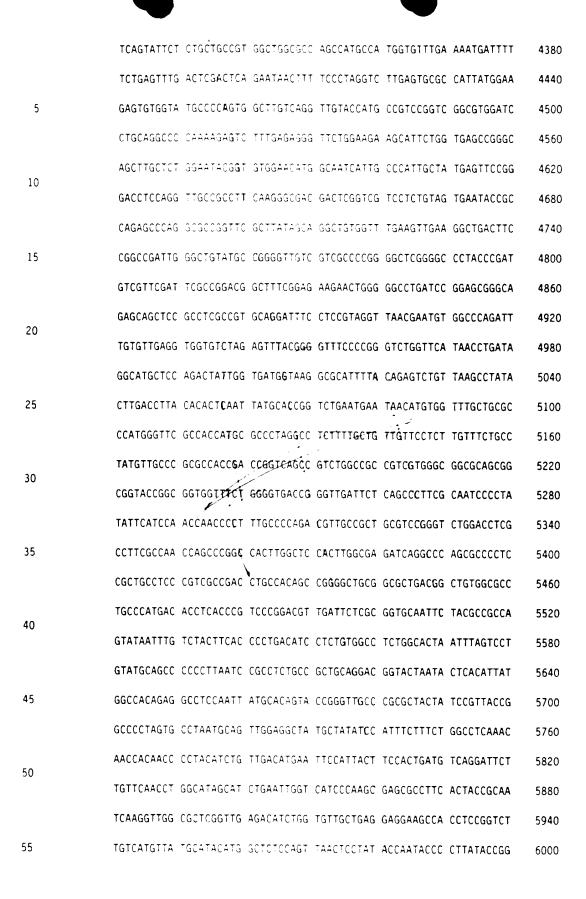
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30	(CATCGGGTC	GCCATTTCAA	CCTATACCAC	CAGGCTTGGG	GCCGGTCCGG	TCGCCATTTC	6900
	Ţ	GCGGCCGCG	GTTTTGGCTC	CACGCTCCGC	ccreecters	CTGGAGGATA	CTTTTGATTA	6960
	T	CCGGGGCGG	GCGCACACAT	TTGATGACTT	CTGCCCTGA	TGCCGCGCTT	TAGGCCTCCA	7020
35	G	GGTTGTGCT	TTCCAGTCA	CTGTCGCTGA	GCTCCAGCGC ¹	CTTAAAGTTA	AGGTGGGTAA	7080
	A	ACTCGGGAG	TTGTAGTTTA	TTTGGCTGT G	CCCACCTACT	TATATCTGCT	GATTTCCTTT	7140
40	А	TTTCCTTTT	TCTCGGTCCC	GCGCTCCCTG	A .			7171
				SEQ ID		1		
				#CCTGTGAGT				60
45				GTCCTT3AGC				120
				TGCAATAAGT				180
	A.	AGTGGGCCA	GGGCATTTCG	GCCTGGAGTA	ASACCTTCTG	TGCCCTTTTC	GGCCCCTGGT	240
50	T	CCGTGCTAT	TGAGAAGGCT	ATTCTGGCCC	TGCTCCCTCA	GGGTGTGTTT	TATGGGGATG	300
	C	CTTTGATGA	CACCGTCTTC	TCGGCGCGTG	TGGCCGCAGC	AAAGGCGTCC	ATGGTGTTTG	360
55	A	GAATGACTT	TTCTGAGTTT	SACTOCACCO	AGAATAATTT	TTCCCTGGGC	CTAGAGTGTG	420
	C.	TATTATGGA	GAAGTGTGGG	ATGOOG44GT	GGCTCATCCG	CTTGTACCAC	CTTATAAGGT	480

	CTGCGTGGAT	CCTGCAGGCC	CCGAAGGAGT	CCCTGCGAGG	GTGTTGGAAG	AAACACTCCG	540
5	GTGAGCCCGG	CACTCTTCTA	TGGAATACTG	TCTGGAACAT	GGCCGTTATC	ACCCATTGTT	600
J	ACGATTTCCG	CGATTTGCAG	GTGGCTGCCT	TTAAAGGTGA	TGATTCGATA	GTGCTTTGCA	30ر
	GTGAGTACCG	TCAGAGTCCA	GGGGCTGCTG	TCCTGATTGC	TGGCTGTGGC	TTAAAGCTGA	720
10	AGGTGGGTTT	CCGTCCGATT	GGTTTGTATG	CAGGTGTTGT	GGTGACCCCC	GGCCTTGGCG	780
	CGCTTCCCGA	CGTCGTGCGC	TTGTCCGGCC	GGCTTACTGA	GAAGAATTGG	GGCCCTGGCC	840
15	CTGAGCGGGC	GGAGCA G CTC	CGCCTTGCTG	TGCG			874
12		.1	+ h	^+ ^			

- or a sequence complementary thereto.
- 4. A protein which is (a) immunoreactive with antibodies present in individuals infected with enterically transmitted nonA/nonB hepatitis and (b) derived from a viral hepatitis agent whose genome contains a region which is homologous to the 1.33 kb DNA EcoRI insert present in plasmid pTZXFl(ET1.1) carried in E. coli strain BB4, and having ATCC Deposit Nno. 67717.
 - 5. The protein of claim 4, which is encoded by a coding region within said 1.33 kb EcoRI insert.
- 6. A protein which is (a) immunoreactive with antibodies present in individuals infected with enterically transmitted nonA/nonB hepatitis and (b) encoded by genetic sequence 406.3-2 or 406.4-2 or a fragment thereof.

- 7. A method of detecting infection by enterically transmitted nonA/nonB hepatitis viral agent in a test individual, comprising:
- providing a peptide antigen which is (a)

 immunoreactive with antibodies present in individuals infected with enterically transmitted nonA/nonB hepatitis and (b) derived from a viral hepatitis agent whose genome contains a region which is homologous to

the 1.33 kb DNA ECORI insert present in plasmid pTZKF1(ET1.1) carried in E. coli strain BB4, and having ATCC deposit no. 67717,

5

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reacting serum from the test individual with such antigen, and

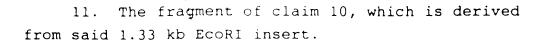
examining the antigen for the presence of bound antibody.

- 8. The method of claim 7, wherein the serum
 antibody is an IgM or IgG antibody, or a mixture of
 both, the antigen provided is attached to a support,
 said reacting includes contacting such serum with the
 support and said examining includes reacting the
 support and bound serum antibody with a reporterlabeled anti-human antibody.
 - 9. A kit for accertaining the presence of serum antibodies which are diagnostic of enterically transmitted nonA/nonB hepatitis infection, comprising

a support with surface-bound recombinant peptide antigen which is (a) immunoreactive with antibodies present in individuals infected with enterically transmitted nonA/nonB viral hepatitis agent and (b) derived from a viral hepatitis agent whose genome contains a region which is homologous to the 1.33 kb DNA EcoRI insert present in plasmid pTZKF1(ET1.1) carried in <u>E. coli</u> strain BB4, and having ATCC deposit no. 67717, and

a reporter-labeled anti-human antibody.

10. A DNA fragment derived from an enterically transmitted nonA/nonB viral hepatitis agent whose genome contains a region which is homologous to the 1.33 kb DNA EcoRI insert present in plasmid pTZKF1(ET1.1) carried in \underline{E} . \underline{coli} strain BB4 and having ATCC deposit no. 67717.

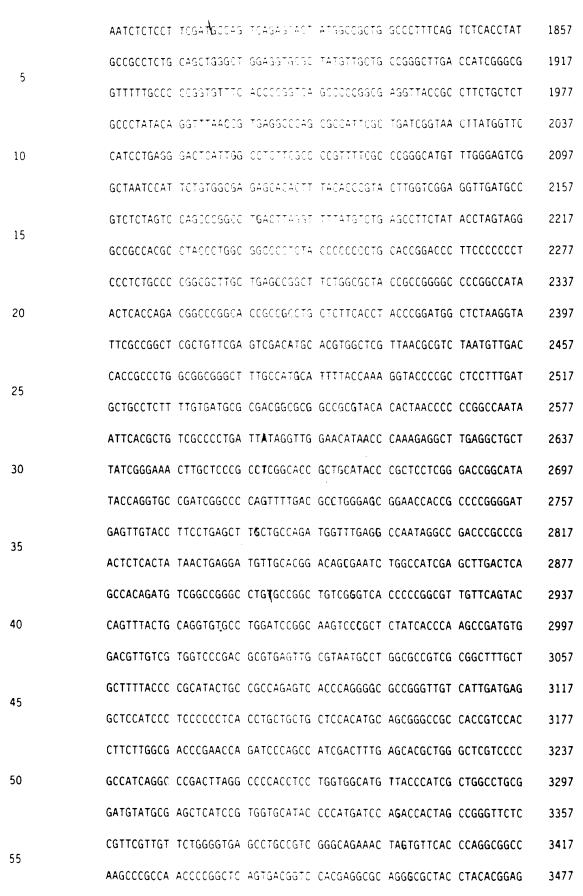


- 12. A DNA molecule comprising genetic sequence
 5 406.3-2 or 406.4-2 or a fragment thereof, wherein said
 fragment comprises at least 12 consecutive
 nucleotides.
- 13. A DNA fragment derived from an enterically transmitted nonA/nonB viral hepatitis agent whose genome contains a region which is homologous to a DNA fragment within a first sequence (SEQ ID NO.1):

AGACCTGTCC CTGTTGCAGC TGTTCTACCA CCCTGCCCCG AGCTCGAACA GGGCCTTCTC 60 15 TACCTGCCCC AGGAGCTCAC CACCTGTGAT AGTGTCGTAA CATTTGAATT AACAGACATT 120 GTGCACTGCC GCATGGCCGC CCCGAGCCAG CGCAAGGCCG TGCTGTCCAC ACTCGTGGGC 180 CGCTACGGCG GTCGCACAAA GCTCTACAAT GCTTCCCACT CTGATGTTCG CGACTCTCTC 240 20 GCCCGTTTTA TCCCGGCCAT TGGCCCCGTA CAGGTTACAA CTTGTGAATT GTACGAGCTA 300 GTGGAGGCCA TGGTCGAGAA GGGCCAGGAT GGCTCCGCCG TCCTTGAGCT TGATCTTTGC 360 25 AACCGTGACG TGTCCAGGAT CACCTTCTTC CAGAAAGATT GTAACAAGTT CACCACAGGT 420 GAGACCATTG CCCATGGTAA AGTGGGCCCAG GGCATCTCGG CCTGGAGCAA GACCTTCTGC 480 GCCCTCTTTG GCCCTTGGTT CCGCGCTATT GAGAAGGCTA TTCTGGCCCT GCTCCCTCAG 540 30 GGTGTGTTTT ACGGTGATGC CTTTGATGAC ACCGTCTTCT CGGCGGCTGT GGCCGCAGCA 600 AAGGCATCCA TGGTGTTTGA GAATGACTTT TCTGAGTTTG ACTCCACCCA GAATAACTTT 660 35 TCTCTGGGTC TAGAGTGTGC TATTATGGAG GAGTGTGGGA TGCCGCAGTG GCTCATCCGC 720 CTGTATCACC TTATAAGGTC TGCGTGGATC TTGCAGGCCC CGAAGGAGTC TCTGCGAGGG 780 TTTTGGAAGA AACACTCCGG TGAGCCCGGC ACTCTTCTAT GGAATACTGT CTGGAATATG 840 40 GCCGTTATTA CCCACTGTTA TGACTTCCGC GATTTTCAGG TGGCTGCCTT TAAAGGTGAT 900 GATTCGATAG TGCTTTGCAG TGAGTATCGT CAGAGTCCAG GAGCTGCTGT CCTGATCGCC 960 GGCTGTGGCT TGAAGTTGAA GGTAGATTTC CGCCCGATCG GTTTGTATGC AGGTGTTGTG 45 1020 GTGGCCCCG GCCTTGGCGC GCTCCCTGAT GTTGTGCGCT TCGCCGGCCG GCTTACCGAG 1080 AAGAATTGGG GCCCTGGCCC TGAGCGGGCG GAGCAGCTCC GCCTCGCTGT TAGTGATTTC 1140 50 96

	CTCCGCAAGO TCACGAATGT AGCTCAGATG TGTGTGGATG TTGTTTCCCG TGTTTATGGG	1200			
	GTTTCCCCTG SACTEGTTCA TAACCTGATT GGCATGCTAC AGGCTGTTGC TGATGGCAAG	1260			
5	GCACATTTCA CTGAGTCAGT AAAAACCASTS CTCGA	1295			
	a second sequence (SEQ ID NO.5):				
	TOGAGOACTG STETTACTGA CTCAGTSAAA TGTGCCTTGC CATCAGCAAC AGCCTGTAGC	60			
10	ATGCCAATCA GGTTATGAAC GAGTSCAGGG GAAAGGSCCAT AAACACGGGA AACAACATCC	120			
	ACACACATOT BAGGTACATT CGTGAGGTTG CGGAGGAAAT CACTAACAGC GAGGCGGAGC	180			
15	TGCTCCGCCC GCTCAGGGCC AGGGCCCCAA TTCTTCTCGG TAAGCCGGCC GGCGAAGCGC	240			
15	ACAACATCAG GGAGCGCGCC AAGGCCGGGG GCCACCACAA CACCTGCATA CAAACCGATC	300			
	GGGCGGAAAT CTACCTTCAA CTTCAAGCCA CAGCCGGCGA TCAGGACAGC AGCTCCTGGA	360			
20	CTCTGACGAT ACTCACTGCA AAGCACTATO GAATCATCAC CTTTAAAGGC AGCCACCTGA	420			
	AAATCGCGGA AGTCATAACA GTGGGT#ATA ACGGCCATAT TCCAGACAGT ATTCCATAGA	480			
25	AGAGTGCCGG GCTCACCGGA GTGTTTCTTC CAAAACCCTC GCAGAGACTC CTTCGGGGCC	540			
25	TGCAAGATCC ACGCAGACCT TATAAGGTGA TACAGGCGGA TGAGCCACTG CGGCATCCCA	600			
	CACTCCTCCA TAATAGCACA CTCTAGACCC AGAGAAAAGT TATTCTGGGT GGAGTCAAAC	660			
30	TCAGAAAAGT CATTCTCAAA CACCATGGAT GCCTTTGCTG CGGCCACAGC CGCCGAGAAG	720			
	ACGGTGTCAT CAAAGGCATC ACCGTAAAAC ACACCCTGAG GGAGCAGGGC CAGAATAGCC	780			
25	TTCTCAATAG CGCGGAACCA AGGGCCAAAG AGGGCGCAGA AGGTCTTGCT CCAGGCCGAG	840			
35	ATGCCCTGGC CCACTITACC ATGGGCAATG GTCTCACCTG TGGTGAACTT GTTACAATCT	900			
	TTCTGGAAGA AGGTGATCCT GGACACGTCA CGGTTGCAAA GATCAAGCTC AAGGACGGCG	960			
40	GAGCCATCCT GGCCCTTCTC GACCATGGCC TCCACTAGCT CGTACAATTC ACAAGTTGTA	1020			
	ACCTGTACGG GGCCAATGGC CGGGATAAAA CGGGCGAGAG AGTCGCGAAC ATCAGAGTGG	1080			
A.F.	GAAGCATTGT AGAGCTTTGT GCGACCGCCG TAGCGGCCCA CGAGTGTGGA CAGCACGGCC	1140			
45	TTGCGCTGGC TCGGGGCGGC CATGCGGCAG TGCACAATGT CTGTTAATTC AAATGTTACG	1200			
	ACACTATCAC AGGTGGTGAG CTCCTGGGGC AGGTAGAGAA GGCCCTGTTC GAGCTCGGGG	1260			
50	CAGGGTGGTA GAACAGCTGC AACAGGGACA GGTCT	1295			
	a third sequence (SEQ ID NO.6):				
	AGGCAGACCA CATATGTGGT CGATGCC ATGGAGGCCC ATCAGTTTAT TAAGGCTCCT	57			
55	GGCATCACTA CTGCTATTGA GCAGGCTGCT CTAGCAGCGG CCAACTCTGC CCTGGCGAAT	117			

	GCTGTGGTAG	S TTAGGOSTIT	7676767646	CAGCAGATTG	AGATCCTCAT	TAACCTAATG	177
5	CAACCTCGCC	CAGCTTGTTTT	CCGCCCCGAG	GTTTTCTGGA	ATCATCCCAT	CCAGCGTGTC	237
J	ATCCATAACC	AGCTGGAGCT	TTACTGCCGC	SCCCGCTCCG	GCCGCTGTCT	TGAAATTGGC	297
	GCCCATCCCC) GCTCAATAAA	TGATAATOCT	AATGTGGTCC	ACCGCTGCTT	CCTCCGCCCT	357
10	GTTGGGCGTG	ATSTTCAGCG	CIGGTATACT	GCTCCCACTC	GCGGGCCGGC	TGCTAATTGC	417
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15	TCTGGCTGTA	ACTITICCGC	CGAGACTGGC	ATCGCCCTCT	ACTCCCTTCA	TGATATGTCA	537
15	CCATCTGATG	TCGCCGAGGC	CATGTTCCGC	CATGGTATGA	CGCGGCTCTA	TGCCGCCCTC	597
	CATCTTCCGC	CTGAGGTCCT	GCTGCCCCCT	GGCACATATC	GCACCGCATC	GTATTTGCTA	657
20	ATTCATGACG	GTAGGCGCGT	TGTGGTGACG	TATGAGGGTG	ATACTAGTGC	TGGTTACAAC	717
	CACGATGTCT	CCAACTTGCG	CTCCTGGATT	AGAACCACCA	AGGTTACCGG	AGACCATCCC	777
25	CTCGTTATCG	AGCGGGTTAG	GGCCATTGGC	TGCCACTTTG	TTCTCTTGCT	CACGGCAGCC	837
23	CCGGAGCCAT	CACCTATGCC	TTATGTTCCT	TACCCCCGGT	CTACCGAGGT	CTATGTCCGA	897
	TCGATCTTCG	GCCCGGGTGG	CACCCCTTCC	TTATTCCCAA	CCTCATGCTC	CACTAAGTCG	957
30	ACCTTCCATG	статссстас	CCATATTTGG	GACCGTCTTA	TGCTGTTCGG	GGCCACCTTG	1017
	GATGACCAAG	CCTTTTGCTG	CT CC CGTTTA	ATGACCTACC	TTCGCGGCAT	TAGCTACAAG	1077
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55	ACAGTAAAGG	TCTCCCAGGT	CGATGGGCGG	ATCGATTGCG	AGACCCTTCT	TGGTAACAAA	1737
J J	ACCTTTCGCA	CGTCGTTCGT	TGACGGGGGG	GTCTTAGAGA	CCAATGGCCC	AGAGCGCCAC	1797



	(

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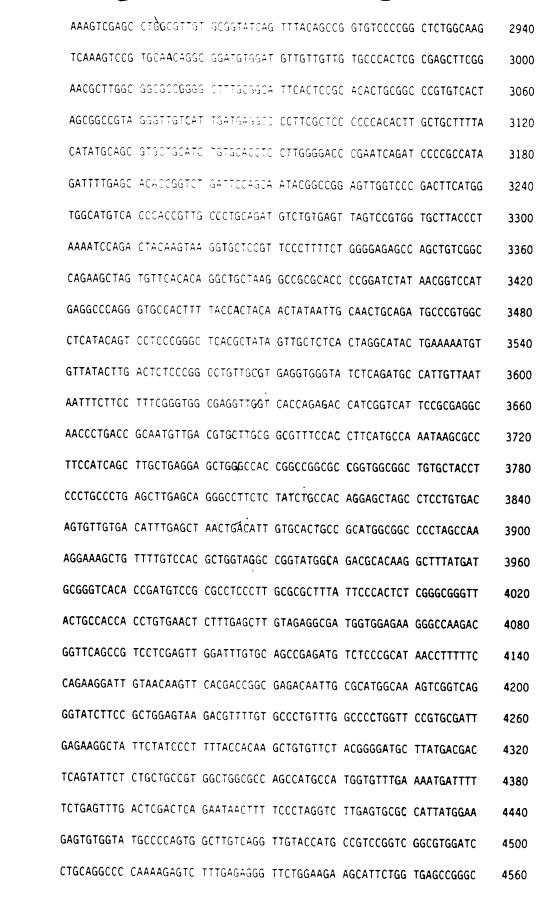




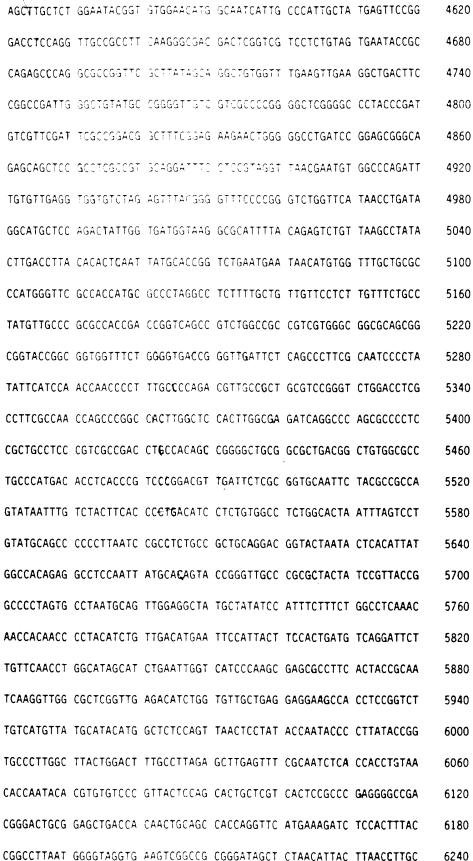
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15	a fourth sequence (SEQ ID NO.10):						
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	CCTAATGTCC TCCATCGCTG CTTTCTCCAC CCCGTCGGCC GGGATGTTCA GCGCTGGTAC	360					
30	ACAGCCCCGA CTAGGGGACC TGCGGCGAAC TGTCGCCGCT CGGCACTTCG TGGTCTGCCA	420					
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	CCTTACCCGC GTTCGACGGA GGTCTATGTC CGGTCTATCT TTGGGCCCGG CGGGTCCCCG	900					
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	CTTATGACGT ACCTTCGTGG CATTAGCTAT AAGGTAACTG TGGGTGCCCT GGTCGCTAAT	1080					
	GAAGGCTGGA ATGCCACCGA GGATGCGCTC ACTGCAGTTA TTACGGCGGC TTACCTCACA	1140					
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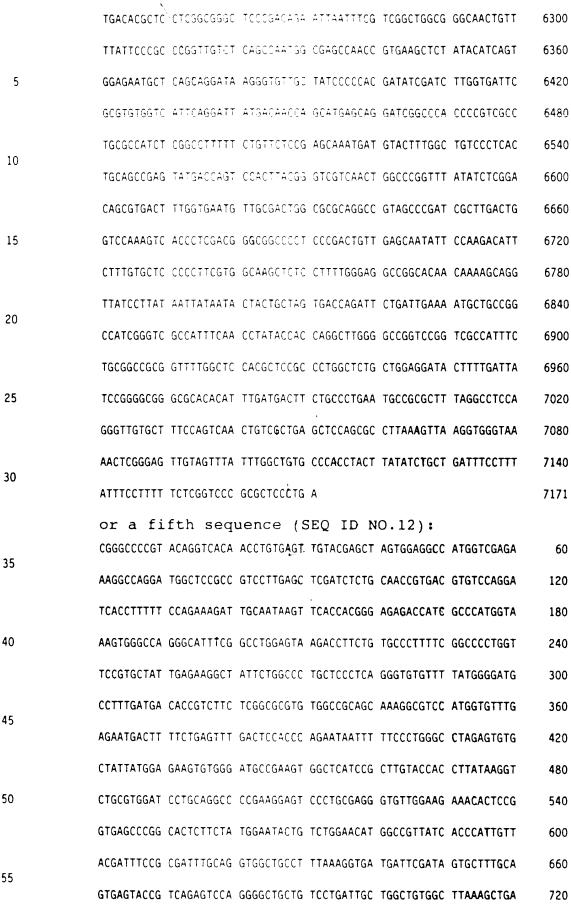


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20	GCACGCCTTG AGGTTAACGG GCCTSAGCAG CTTAACCTCT CTTTTGACAG CCAGCAGTGT	1800
	AGTATGGCAG CCGGCCCGTT TTGCCTCACC TATGCTGCCG TAGATGGCGG GCTGGAAGTT	1860
	CATTITICCA COGCIGGOOT CGAGAGOOGT GIFGITITOO COCCIGGIAA IGCCCCGACT	1920
25	GCCCCGCCGA GTGAGGTCAC CGCCTTCTGC TCAGCTCTTT ATAGGCACAA CCGGCAGAGC	1980
	CAGCGCCAGT CGGTTATTGG TAGTTTGTGG CTGCACCCTG AAGGTTTGCT CGGCCTGTTC	2040
30	CCGCCCTTTT CACCCGGGCA TGAGTGGCGG TCTGCTAACC CATTTTGCGG CGAGAGCACG	2100
	CTCTACACCC GCACTTGGTC CACAATTACA GACACCCT TAACTGTCGG GCTAATTTCC	2160
	GGTCATITGG ATGCTGCTCC CCACTCGGGG GGGCCACCTG CTACTGCCAC AGGCCCTGCT	2220
35	GTAGGCTCGT CTGACTCTCC AGACCCTGAC CCGCTACCTG ATGTTACAGA TGGCTCACGC	2280
	CCCTCTGGGG CCCGTCCGGC TGGCCCCAAC CCGAATGGCG TTCCGCAGCG CCGCTTACTA	2340
40	CACACCTACC CTGACGGCGC TAAGATCTAT GTCGGCTCCA TTTTCGAGTC TGAGTGCACC	2400
	TGGCTTGTCA ACGCATCTAA CGCCGGCCAC CGCCCTGGTG GCGGGCTTTG TCATGCTTTT	2460
	TTTCAGCGTT ACCCTGATTC GTTTGAEGEC ACCAAGTTTG TGATGCGTGA TGGTCTTGCC	2520
45	GCGTATACCC TTACACCCCG GCCGATCATT CATGCGGTGG CCCCGGACTA TCGATTGGAA	2580
	CATAACCCCA AGAGGCTCGA GGCTGCCTAC CGCGAGACTT GCGCCCGCCG AGGCACTGCT	2640
50	GCCTATCCAC TCTTAGGCGC TGGCATTTAC CAGGTGCCTG TTAGTTTGAG TTTTGATGCC	2700
	TGGGAGCGGA ACCACCGCCC GTTTGACGAG CTTTACCTAA CAGAGCTGGC GGCTCGGTGG	2760
		2820
55	GCCAACCTGG CCCTGGAGCT TGACTCCGGG AGTGAAGTAG GCCGCGCATG TGCCGGGTGT	2880









AGGTGGGTTT	CCGTCCGATT	GGTTTST4T3	CAGGTGTTGT	GGTGACCCCC	GGCCTTGGCG	780
CGCTTCCCGA	CGTCGTGCGC	TTGTCCGGCC	GGCTTACTGA	GAAGAATTGG	GGCCCTGGCC	840
CTGAGCGGGC	GGAGCAGCTC	CGCCTTGCTG	TGCG			874

or a sequence complementary thereto.

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- 14. A kit comprising, in a container or separate containers, a pair of single-strand primers derived from nonhomologous regions of opposite strands of a DNA duplex fragment derived from an enterically transmitted viral hepatitis agent whose genome contains a region which is homologous to the 1.33 kb DNA EcoRI insert present in plasmid pTZKF1(ET1.1) carried in E. coli strain BB4 and having ATCC deposit no. 67717.
- 20 15. The kit of claim 15, which are derived from opposite strands of the EcoRI duplex insert in said plasmid.
- 16. A method for detecting the presence of an enterically transmitted nonA/nonB hepatitis viral agent in a biological sample, comprising

preparing a mixture of duplex DNA fragments derived from the sample,

denaturing the duplex fragments,

adding to the denatured DNA fragments, a pair of single-strand primers derived from nonhomologous regions of opposite strands of a DNA duplex fragment derived from an enterically transmitted viral hepatitis agent whose genome contains a region which is homologous to the 1.33 kb DNA EcoRI insert present in plasmid pTZKF1(ET1.1) carried in E. coli strain BB4, and having ATCC deposit no. 67717,

hybridizing said primers to homologous-sequence region of opposite strands of such duplex DNA

fragments derived from enterically transmitted
nonA/non'B hepatitis agent,

reacting the primed fragment strands with DNA
polymerase in the presence of DNA nucleotides, to form
new DNA duplexes containing the primer sequences, and
repeating said denaturing, adding, hybridizing
and reacting steps, until a desired degree of

amplification of sequences is achieved.

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- 17. The method of claim 16, wherein the primers are derived from opposite strands of the EcoRI duplex insert in said plasmid.
- 18. The method of claim 16, for detecting the presence of viral agent in a sample of cultured cells infected with the agent.
- 19. A vaccine for immunizing an individual against enterically transmitted nonA/nonB hepatitis viral agent comprising, in a pharmacologically acceptable adjuvant, a recombinant protein derived from an enterically transmitted nonA/nonB viral hepatitis agent whose genome contains a region which is homologous to the 1.33 kb DNA EcoRI insert present in plasmid pTZ-RF1(ET1.1) carried in E. coli strain BB4, and having ATCC deposit no. 67717.
 - 20. The vaccine of claim 19, wherein the protein is derived from the EcoRI insert in said plasmid.
 - 21. A vaccine for immunizing an individual against HEV comprising, in a pharmacologically acceptable adjuvant, a protein encoded by genetic sequence 406.3-2 or 406.4-2 or a fragment thereof.
 - 22. In a method of isolating an enterically transmitted nonA/nonB viral agent or a nucleic acid fragment produced by the agent, an improvement which

comprises: utilizing, as a source of said agent, bile obtained from a human or cynomolgus monkey having an active infection of enterically transmitted non-A/non-B hepatitis.

23. The method of claim 22, wherein the bile is obtained from an infected cynomolgus monkey.

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24. Human polyclonal anti-serum obtained from a human immunized with a protein derived form an enterically transmitted non-A/non-B viral hepatitis agent whose genome contains a region which is homologous to the 1.33 kb DNA EcoRI insert present in plasmid pTZKF1(ET1.1) carried in E. coli strain BB4 and having ATCC deposit no. 67717.